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APPLICATION NUMBER: *60/513,411*
FILING DATE: *October 22, 2003*
RELATED PCT APPLICATION NUMBER: *PCT/US04/35295*

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Jon W Dudas

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION under 37 CFR 1.53(c).

Docket Number:	23540-08548

INVENTOR(s)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City And Either State Or Foreign Country)
Bing	Guo	Woodland, California
Ian M.	Kennedy	Davis, California
Dosi	Dosev	Davis, California
Darina	Melnikov	Carmichael, California

☐ Additional inventors are being named on one separately numbered sheet attached hereto.

TITLE OF THE INVENTION (500 characters max.)

Methods for Preparing and Functionalizing Nanoparticles

CORRESPONDENCE ADDRESS

Direct all correspondence to:

☒ Customer Number 00758

ENCLOSED APPLICATION PARTS (check all that apply)

☒	Specification	*No. of Pages:* 17	☒	Return Postcard
☒	Drawing(s)	*No.of Sheets:* 10	☐	CD(s), Number
☒	Application Data Sheet See 37 CFR 1.76		☐	Other *(specify)*

METHOD OF PAYMENT (check all that apply)

☐ Applicant claims small entity status. See 37 CFR 1.27

☒ Fee Transmittal Form Enclosed (in duplicate) ☐ Check Enclosed

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ Yes, the name of the U.S. Government Agency and the Government contract number are: Grant No. 5P42ES04699 awarded by the National Institutes of Health and Grant No. 0102662 awarded by the National Science Foundation.

SIGNATURE OF ATTORNEY OR AGENT

Signature:	Michael J. Shuster		
Attorney/Reg. No.:	Michael J. Shuster, Reg. No. 41,310	Dated:	Oct. 22, 2003

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service pursuant to 37 CFR 1.10 in an envelope addressed to: Mail Stop Provisional Patent Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date shown below.

Signature:	Michael J. Shuster		
Typed or Printed Name:	Michael J. Shuster	Dated:	Oct. 22, 2003
Express Mail Mailing Number:	EV 338362427 US		

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17224
102203

FEE TRANSMITTAL for FY 2004

Patent fees are subject to annual revision.

☒ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 80.00

Complete if Known	
Application Number	New Application
Filing Date	October 22, 2003
First Named Inventor	Bing Guo, et al.
Examiner Name	Not Yet Known
Art Unit	Not Yet Known
Attorney Docket No.	23540-08548

15757 U.S. PTO 60/513411 10/22/03

METHOD OF PAYMENT (check all that apply)

☐ Check ☐ Credit Card ☐ Money Order ☐ Other ☐ None

☒ Deposit Account:

Deposit Account Number: 19-2555

Deposit Account Name: Fenwick & West LLP

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FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1001	770	2001	385	Utility filing fee	
1002	340	2002	170	Design filing fee	
1003	530	2003	265	Plant filing fee	
1004	770	2004	385	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	80

SUBTOTAL (1) ($) 80

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

		Extra Claims		Fee from below		Fee Paid
Total Claims	-20** =		X		=	
Independent Claims	-3** =		X		=	
Multiple Dependent					=	

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	88	2201	43	Independent claims in excess of 3
1203	290	2203	145	Multiple dependent claim, if not paid
1204	86	2204	43	**Reissue independent claims over original patent
1205	18	2205	9	**Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($) 0.00

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FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1051	130	2051	65	Surcharge - late filing fee or oath	
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for *ex parte* reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	420	2252	210	Extension for reply within second month	
1253	950	2253	475	Extension for reply within third month	
1254	1,480	2254	740	Extension for reply within fourth month	
1255	2,010	2255	1,005	Extension for reply within fifth month	
1401	330	2401	165	Notice of Appeal	
1402	330	2402	165	Filing a brief in support of an appeal	
1403	290	2403	145	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive - unavoidable	
1453	1,330	2453	665	Petition to revive - unintentional	
1501	1,330	2501	665	Utility issue fee (or reissue)	
1502	480	2502	240	Design issue fee	
1503	640	2503	320	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17(g)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	770	2809	385	Filing a submission after final rejection (37 CFR 1.129(a))	
1810	770	2810	385	For each additional invention to be examined (37 CFR 1.129(b))	
1801	770	2801	385	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	

Other fee (specify) _____

SUBTOTAL (3) ($) 0.00

*Reduced by Basic Filing Fee Paid

SUBMITTED BY

Name (Print/Type)	Michael J. Shuster	Registration No. (Attorney/Agent)	41,310	Complete (if applicable) Telephone (415) 875-2413
Signature	Michael J. Shuster	Date	Oct. 22, 2003	

FEE TRANSMITTAL for FY 2004

Patent fees are subject to annual revision.

☒ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 80.00

Complete if Known	
Application Number	New Application
Filing Date	October 22, 2003
First Named Inventor	Bing Guo, et al.
Examiner Name	Not Yet Known
Art Unit	Not Yet Known
Attorney Docket No.	23540-08548

METHOD OF PAYMENT (check all that apply)

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☒ Deposit Account:

Deposit Account Number: 19-2555

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2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

	Extra Claims		Fee from below		Fee Paid
Total Claims		-20** =		X	=
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SUBTOTAL (2) ($) 0.00

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FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1051	130	2051	65	Surcharge - late filing fee or oath	
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SUBMITTED BY

Complete (if applicable)

Name (Print/Type)	Michael J. Shuster	Registration No. (Attorney/Agent)	41,310	Telephone (415) 875-2413
Signature	Michael J. Shuster	Date	Oct. 22, 2003	

PROVISIONAL APPLICATION FOR UNITED STATES PATENT

METHODS FOR PREPARING AND FUNCTIONALIZING NANOPARTICLES

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Filing Date:	October 22, 2003
Express Mail No:	EV 338362427 US

TITLE

[0001] Methods for Preparing and Functionalizing Nanoparticles.

CROSS REFERENCE TO RELATED APPLICATIONS

[0002] Not applicable.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0003] The U.S. Government has certain rights in this invention pursuant to Grant No. 5P42ES04699 awarded by the National Institutes of Health and Grant No. 0102662 awarded by the National Science Foundation.

BACKGROUND OF THE INVENTION

Field of the invention

[0004] This invention relates to the fields of chemistry and biology.

Description of the Related Art

[0005] Fluorescence is a widely used tool in chemistry and biological science. Fluorescent labeling of molecules is a standard technique in biology. The labels are often organic dyes that give rise to the usual problems of broad spectral features, short lifetime, photobleaching, and potential toxicity to cells. Alternative labels may be based on lanthanide-derived phosphors. The recent emerging technology of quantum dots has spawned a new era for the development of fluorescent labels using inorganic complexes or particles. These materials offer substantial advantages over organic dyes including larger Stokes shift, longer emission half-life, narrow emission peak and minimal photo-bleaching. However, quantum dot technology still is in its infancy, and is plagued by many problems including difficulties

associated with reproducible manufacture, coating, and derivatization of quantum dot materials.

[0006] In addition, although the quantum yield of an individual quantum dot is high, the actual fluorescence intensity of each tiny dot is low. Grouping multiple quantum dots into larger particles is one approach for increasing the fluorescence intensity, but this nascent technology still suffers from drawbacks including difficulties in generating and maintaining uniform particle size distributions. Wider application of quantum dot technology therefore has been limited by the difficulties referred to above.

[0007] Rare-earth metal elements such as europium are known for their unique optical (fluorescent/phosphorescent) properties. When their salts are dissolved in water, their fluorescence is quenched. Thus, many investigators have used europium and other rare-earth chelates to label biological molecules for the sensitive detection of proteins and nucleic acids, to carry out time-resolved fluorometric assays, and as labels in immunoassays. However, this chelation chemistry often is expensive and complex, and so application of rare-earth chelation technology also has been limited to date.

[0008] Recently, nanoparticles have received much attention in biology. These particles can have strong fluorescence that exhibits a spectrally sharp emission peak, large Stokes shift, and less quenching influence by other chemicals. Nanoparticles such as Eu_2O_3 particles also have been recognized as offering tremendous potential in obtaining large enhancement of emission intensity. However, Eu_2O_3 and other nanoparticles are easily dissolved by acid during activation and conjugation, thereby losing their desirable properties. In addition, nanoparticles lack reactive groups that allow them to be easily derivatized and linked to analytes and other reagents, thus increasing the difficulty associated with using nanoparticles as labeling reagents for the study of biological and other molecules.

[0009] Silica and alumina surfaces have wide-ranging surface reactivities; in particular, silica can be used as a cap to keep europium oxide from dissolving in acid in the conjugation process. However, coating with silica and alumina may increase the particle size, thereby compromising the advantageous properties of nanoparticles that render them suitable as labeling reagents.

[0010] The present invention addresses these and other limitations of the prior art by providing methods for manufacturing and derivatizing nanoparticles, and derivatized nanoparticle compositions that retain the optical properties of the native particles and enable the efficient and low-cost use of the nanoparticles to label biological and other materials.

SUMMARY OF THE INVENTION

[0011] The present invention is defined by the following claims, and nothing in this section should be taken as a limitation on those claims. Disclosed herein are gas-phase flame synthesis methods, apparatus for their synthesis, nanoparticle compositions as well as methods for functionalizing nanoparticles.

[0012] Accordingly one aspect of the invention includes a silica glass nanoparticle, co-doped with a rare earth element and another metal element. In one aspect, the invention includes an apparatus for preparing said nanoparticles by gas-phase combustion and/or pyrolysis synthesis. In another aspect, the invention includes a method for preparing nanoparticles using a gas-phase combustion and/or pyrolysis synthesis. The method comprises a gas-phase flame synthesis process in which, a lanthanide compound, optionally another metal and optionally a silicon compound are introduced into a flame by entraining the vapor or atomized spray of said materials with a gaseous fuel or entraining the vapor or atomized spray of said materials in a separate gas that mixes with the gaseous fuel prior to entering a reaction zone in which the flame is present. In the reaction zone, the reactants undergo decomposition and/or

oxidation reactions to form the corresponding oxides. The hot vapor or atomized spray of the oxides nucleate and condense at lower temperatures to form solid particles. The particles are collected and may be subject to further treatment. The chemical composition of the resulting particles and their physical attributes such as size and shape are controlled by adjusting the relative concentrations of each precursor. In another aspect the invention includes a method for functionalizing nanoparticles by mixing a functionalizing agent vapor with a humidified aerosol comprising the nanoparticles. Water molecules present on the surface of the nanoparticles facilitates the coating reaction, which results in a layer of free reactive chemical groups on the surface of the particles. The reactive groups permit the particles to be conjugated with, e.g., molecules of biological interest such as proteins, carbohydrates, and nucleic acids. The aerosol containing the particles is introduced in a reaction chamber in which it joins a steady flow of functionalizing agent vapor that may optionally be entrained in an inert carrier gas. The functionalization reaction takes place on the surface of the particles while they are suspended in the reaction chamber. These methods largely avoid the agglomeration problems encountered with liquid-phase functionalization reactions and also greatly reduce or eliminate the need of post-functionalization washing of the particles.

[0013] In one embodiment, the compositions of the invention comprise silica, the lanthanide is europium and the at least one other metal is sodium. In a preferred embodiment of the functionalization methods, the functionalization reagent is a silane. Exemplary embodiments include 3-aminopropyltriethoxysilane, 3-aminopropyltrimethoxysilane, as well as mixtures of these or other silanes.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWING

[0014] These and other features, aspects, and advantages of the present invention will become better understood with regard to the following description, and accompanying drawings, where:

[0015] **Figure 1** is a schematic of an apparatus for gas phase synthesis of nanoparticles.

[0016] **Figure 2** is a schematic of a pneumatic nebulizer and optional co-flow jacket used in conjunction with the apparatus illustrated in Fig. 1.

[0017] **Figure 3** is a schematic of an apparatus for functionalizing aerosolized nanoparticles.

[0018] **Figure 4** is a transmission electron micrograph of Eu- and Na-doped silica nanoparticle (left side of figure); right side of Fig. 4 illustrates fluorescence emission spectra for Eu-SiO_2 nanoparticles excited at 466 nm (top) and at 532 nm (bottom) showing fluorescence lifetime on order of 2 msec.

[0019] **Figure 5** (left side of figure)is a transmission electron micrograph of EuSi:ZnO nanoparticles; right side of Fig. 5 illustrates fluorescence emission spectra for EuSi:ZnO nanoparticles excited at 532 nm (middle panel) and at 466 nm (right panel) showing fluorescence lifetime on order of 4 msec.

[0020] **Figure 6** is a transmission electron micrograph of Eu_2O_3/SiO_2 nanoparticles (left panel); right panel illustrates fluorescence emission spectrum for Eu_2O_3/SiO_2 nanoparticles excited at 466 nm showing fluorescence lifetime on order of 1 msec.

[0021] **Figure 7** (left panel) is a transmission electron micrograph of pure Eu_2O_3 nanoparticle (monoclinic phase); right panel illustrates fluorescence emission spectrum for pure Eu_2O_3 nanoparticle (monoclinic phase) excited at 466 nm showing short fluorescence lifetime.

[0022] **Figure 8** (left panel) is a transmission electron micrograph of Eu:Y_2O_3 nanoparticle; right panel illustrates fluorescence emission spectrum for pure Eu:Y_2O_3 nanoparticle excited at 260 nm showing fluorescence lifetime on order of 2 msec.

[0023] **Figure 9** illustrates fluorescence emission spectrum of Tb:Y_2O_3 nanoparticle excited at 260 nm showing fluorescence lifetime on order of 2 msec.

[0024] **Figure 10** is a schematic illustrating synthesis, functionalization, and use of nanoparticles in an immunoassay.

DETAILED DESCRIPTION OF THE INVENTION

Advantages and utility

[0025] Briefly, and as described in more detail below, described herein are methods, and apparatus for generating and functionalizing lanthanide-containing nanoparticles.

[0026] Several features of the current approach should be noted. Gas-phase combustion and/or pyrolysis synthesis methods are used for generating lanthanide-containing nanoparticles. In addition, the particles synthesized using the gas-phase combustion and/or pyrolysis synthesis methods may be functionalized to add chemical groups to the surface by mixing a functionalizing agent vapor with a humidified aerosol comprising the nanoparticles.

[0027] Advantages of this approach are numerous. One advantage provided by the invention is a simple and low-cost single-step process to produce nanoparticles that are more uniform and less prone to aggregation than those produced using prior art methods such as ball milling or solution phase syntheses. The functionalization methods disclosed also are simple and low-cost and result in high quality nanoparticles. The functionalization method largely avoids the agglomeration problem encountered with similar procedures that take place in the liquid phase, and greatly reduces or eliminates the need for post-functionalization washing of the nanoparticles.

[0028] The invention provides methods, apparatus and compositions for generating and functionalizing lanthanide-containing nanoparticles that have utility as labels in various applications such as, e.g., immunoassays and nucleic acid based diagnostics.

Definitions

[0029] It must be noted that, as used in the specification, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise.

Materials and methods of the invention

[0030] Table 1 provides a listing of the reagents, abbreviations for the reagents, formulae, suppliers, form of usage of the reagent in the described syntheses and examples of alternative reagents useful for practicing the methods of the invention.

Table 1 – Exemplary Reagents

Reagent	Formula	Supplier	City Supplier Located	Form of Usage in Synthesis	Substitute Reagent
Tris(2,2,6,6-tetramethyl-3,5-heptanedionato) europium(III) abbreviated as $Eu(TMHD)_3$		Alfa Aesar	Ward Hill, MA	Vapor at 200 C	Europium metal, any europium compound that has sufficient vapor pressure at 200 C and does not decompose below 400 C
Sodium metal	Na			Vapor at 400 C	Other alkali or alkaline earth metals
Zinc metal	Zn			Vapor at 400 C	Other alkali or alkaline earth metals
Europium (III)nitrate	$Eu(NO_3)_3.6H_2O$	Alfa Aesar		Aqueous solution or solution in an organic solvent that is readily nebulizable	Other soluble europium salts, such as $EuCl_3$, that does not negatively affect the synthesis reactions

Ytrium (III)nitrate	$Y(NO_3)_3.6H_2O$	Alfa Aesar		Same as above	Other soluble europium salts, such as YCl_3, that does not negatively affect the synthesis reactions
Terbium (III)nitrate	$Tb(NO_3)_3.6H_2O$	Alfa Aesar		Same as above	Other soluble europium salts, such as $TbCl_3$, that does not negatively affect the synthesis reactions
Hexamethyldisiloxane abbreviated as HMDS	$C_6H_{18}OSi_2$	Sigma Aldrich		Both as the vapor and a solution in an organic solvent, such as ethanol, that is readily atomizable	Any other organic compound that contains silicon and has sufficient vapor pressure at room temperature and is soluble in the solvent used for dissolving the other starting materials that does not negatively affect the synthesis reactions

(3-Aminopropyl)triethoxysilane abbreviated as APTES		Sigma	St. Louis, MO	Vapor at room temperature	Many other silanizing reagents.
(3-Aminopropyl)trimethoxysilane abbreviated APTMS		Sigma	St. Louis, MO	Vapor at room temperature	Many other silanizing reagents.

EXAMPLES

[0031] Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperatures, etc.), but some experimental error and deviation should, of course, be allowed for.

[0032] The practice of the present invention will employ, unless otherwise indicated, conventional methods of protein chemistry, biochemistry, recombinant DNA techniques and pharmacology, within the skill of the art. Such techniques are explained fully in the literature. See, e.g., T.E. Creighton, *Proteins: Structures and Molecular Properties* (W.H. Freeman and Company, 1993); A.L. Lehninger, *Biochemistry* (Worth Publishers, Inc., current addition); Sambrook, et al., *Molecular Cloning: A Laboratory Manual* (2nd Edition, 1989); *Methods In Enzymology* (S. Colowick and N. Kaplan eds., Academic Press, Inc.); *Remington's Pharmaceutical Sciences*, 18th Edition (Easton, Pennsylvania: Mack Publishing Company, 1990); Carey and Sundberg *Advanced Organic Chemistry 3rd Ed.* (Plenum Press) Vols A and B(1992).

Methods

[0033] The syntheses have been conducted in a manner that involves a flame as the reaction zone, utilizing an apparatus illustrated in Figure 1 and Figure 2, or a combination of the two.

Functionalization has been carried out using the apparatus illustrated in Figure 3, with an aerosol containing nanoparticles produced by the described syntheses as targets for functionalization.

[0034] Based on the different forms of usage of the starting materials, the syntheses can be divided into two classes, gas-phase synthesis in which all the starting materials are fed into the flame in the vapor phase, and, spray-pyrolysis synthesis in which one or more of the starting materials is fed into the flame in the form of droplets containing the starting material, or solid particles derived from the droplets. The functionalization methods of the present invention may be practiced with nanoparticles synthesized using the disclosed gas-phase combustion and/or pyrolysis synthesis method disclosed herein, or with nanoparticles produced using other manufacturing techniques.

Example 1: Gas-phase synthesis of Eu:Na:Si nanoparticles.

[0035] 50 mg $Eu(TMHD)_3$ and 1 g metal sodium were placed in furnace A shown in Figure 1, in zones at 200° C and 400° C, respectively. Pure H_2 was introduced into furnace A at 0.2 standard Liter/min through the inlet at bottom. Another stream of H_2, after passing through a cartridge containing pure HMDS kept at 23 °C and entraining saturated vapor of HMDS, was also introduced into furnace A. The two streams of H_2 mixed within furnace A and entrained the saturated vapors of the metal sodium and $Eu(TMHD)_3$ at their corresponding temperatures. The H_2 containing all the starting materials was ignited at the outlet of furnace A in 1 atmosphere air. The maximum temperature in the flame was about 2130 °C. The starting materials decomposed in the flame, formed corresponding oxides, and further formed silica glass nanoparticles that contain europium. The particles were determined by transmission electron microscopy to be spherical and not aggregated. Fig. 4, left panel is a transmission electron micrograph showing size and morphology of particles synthesized using

the approach outlined in this example, except that only trace amounts of Eu (carried over from an earlier synthesis) were present. The Eu:Na:Si atomic ratio of the product nanoparticles synthesized in this example was about 1:20:100 as determined by a Philips CM-12 Transmission Electron Microscope equipped with an Oxford Instruments EDX detector for elemental analysis. The particles exhibited strong fluorescence and fluorescence lifetime is about 2 msec. (Data not shown). Right hand panel in Fig. 4 illustrates fluorescence emission spectra for particles synthesized in a manner similar to those described above, except no Na metal was included during the synthesis. Top panel shows emission spectrum using 466 nm excitation wavelength and bottom panel shows emission spectrum using 532 nm excitation wavelength. Fluorescence lifetime was on the order of 2 msec.

[0036] Adjusting the heating temperature for the starting materials that require heating, and the flow rate of the carrier gas for HMDS, allows the fine tuning of the atomic ratios of the elements in the nanoparticles.

Example 2: Gas-phase synthesis of Eu: Zn:Si nanoparticles.

[0037] Methods were the same as those described in Example 1, except that Zn metal was substituted for the Na metal, and trace amounts of Eu were present (carried over from an earlier synthesis). Figure 5 left panel is a transmission electron micrograph illustrating the size and morphology of the nanoparticles made in Example 2. The middle and right hand panels of Fig. 5 illustrate fluorescence emission spectra of the nanoparticles excited at 532 nm (middle panel) and at 466 nm (right hand panel), showing fluorescence lifetime on the order of 4 msec.

Example 3: Gas-phase synthesis of Eu:Si nanoparticles.

[0038] The synthesis conditions were the same as those described in Example 1, except sodium metal was not used. Pure O_2 co-flow was used surrounding the outlet of furnace A, by

mounting an optional co-flow jacket, as shown in Figure 2. The flame temperature was about 2400 °C. A transmission electron micrograph showing the size and morphology of the resulting nanoparticles is shown in the left panel of Figure 6. A fluorescence emission spectrum of the resulting nanoparticles is shown in the right panel of Figure 6. The excitation wavelength was 466 nm, fluorescence lifetime was on the order of 1 msec.

Example 4: Gas-phase synthesis of Eu nanoparticles.

[0039] The synthesis conditions were essentially the same as those described in Example 1, except that only $Eu(TMHD)_3$ was placed in furnace A. The material was heated to 200 °C and entrained in a stream of H_2 gas. The H_2 containing the starting materials was ignited at the outlet of furnace A in 1 atmosphere air. The maximum temperature in the flame was about 2130 °C. The starting material decomposed in the flame, formed the corresponding oxide (i.e., Eu_2O_3). Figure 7, left panel is a transmission electron micrograph of the material synthesized in this example, showing the size and morphology of the nanoparticles. Powder diffraction analysis revealed that the resulting crystals are monoclinic. Right panel of Figure 7 is a fluorescence emission spectrum using an excitation wavelength of 466 nm. The fluorescence lifetime is short due to the small size of the nanoparticles and concentration quenching.

Example 5: Spray-pyrolysis synthesis of Eu:Y nanoparticles.

[0040] An ethanol solution containing 1 mM $Eu(NO_3)_3$ and 30 mM $Y(NO_3)_3$ was pumped with a syringe pump (Cole-Parmer, Vernon Hills, IL) at 7 mL/h into the inner nozzle of the nebulizer illustrated in Figure 2. Ar gas, at 2 standard Liter/min, flowed through the annular gap surrounding the inner nozzle and atomized the ethanol solution containing the starting materials. The solution was atomized to form a spray at the tip of the nebulizer. The nebulizer was combined with an optional co-flow jacket, which supplied H_2 at 2 standard Liter/min and

co-flowed air at 10 standard Liter/min, to form a hydrogen diffusion flame surrounding the outlet of the nebulizer. Flame temperature was about 2100 °C. The H_2 diffusion flame ignited the spray formed by the nebulizer and reactions took place within the flame to form Eu:Y_2O_3 nanoparticles that have desired chemical composition, size and morphology. Figure 8 left panel shows a transmission electron micrograph of the resulting nanoparticles. The right panel of Figure 8 shows a fluorescence emission spectrum using an excitation wavelength of 260 nm. Particles have a fluorescence lifetime on the order of 2 msec.

[0041] In an alternate method, the spray generated by the nebulizer can be introduced into furnace A, along with 2 standard Liter/min H_2. The spray then is preheated in furnace A to remove the solvent from the droplets, to form an aerosol containing dry particles. This aerosol can be ignited at the outlet of furnace A to form a diffusion flame, in which the synthesis reactions take place. Post-synthesis treatment of the nanoparticles produced by the spray-pyrolysis synthesis is optional with furnace B. Post-synthesis treatment helps to remove impurities and improve the crystallographic properties of the nanoparticles formed in the flame. In addition to ethanol, other solvents useful for spray pyrolysis include aqueous ethanol, water, acetone or other lower alcohols, ketones, or any other solvent in which the reagents are stable for the time necessary to carry out the synthesis, and that have a density and molecular weight appropriate to allow atomization of the reagents.

Example 6: Spray-pyrolysis synthesis of Tb:Y nanoparticles.

[0042] Conditions were the same as those described in Example 5, except that $Eu(NO_3)_3$ was replaced by $Tb(NO_3)_3$. The fluorescence emission spectrum of the resulting particles is shown in Fig. 9. Excitation wavelength was 260 nm; fluorescence lifetime was on the order of 2 msec.

Example 7: Functionalization of nanoparticles.

[0043] Functionalization is carried out using the apparatus illustrated in Fig. 3. 4ml of 3-aminopropyltriethoxy-silane (APTES) is contained in a 250 ml Erlenmeyer flask (not shown) having one inlet and one outlet, T=20°C, P= 1atm. Ar gas is used as a carrier gas to deliver APTES vapor into the reaction chamber of Fig. 3. Various flow rates of Ar are used: 50 SCCM, 75 SCCM, 100 SCCM, 150 SCCM.

[0044] The reaction chamber contains two inlets and one outlet. Nanoparticles are collected with a probe located 2-5cm from the burner illustrated in Fig.1. The flow rate of the combustion products gas into the chamber is determined by the vacuum suction rate. In the chamber, APTES vapor mixes with particles. The concentration of water in the aerosol plays an important role in the amino-silane coating of the target nanoparticles within. The presence of water molecule on the surface of the nanoparticles facilitates the binding of the amino-silane molecules with the particles surface. However, excess amounts of water cause cross-linking between the amino-silane molecules and render them useless or even detrimental to the coating process. Hence there is an optimal water vapor concentration for each functionalization process. In the case where nanoparticles are functionalized by coating with (3-Aminopropyl)triethoxysilane freshly from the gas-phase flame synthesis process, the water vapor is originated from the combustion of H_2 and its concentration in the aerosol is adjusted by dilution from the air co-flow assisting the combustion process. The water content in this aerosol is about 0.02 g/Liter, providing effective functionalization of these particles by APTES. The particle concentration in the aerosol is on the order of 106 particles/cm^3, with a typical mean diameter of 50 nm.

[0045] Functionalized particles are collected on the anodisc 47 Whatman filter.

Example 8: Conjugation and use of functionalized nanoparticles.

[0046] Nanoparticles functionalized according to the method described in Example 7 have a free amino group that is used to conjugate the particle to a biomolecule such as an antibody using techniques known to those of ordinary skill in the art. The labeled antibody is used in an immunoassay to detect the presence of an analyte in a sample suspected of containing the analyte. Such methods also are well known to those of ordinary skill in the art.

[0047] While the invention has been particularly shown and described with reference to a preferred embodiment and various alternate embodiments, it will be understood by persons skilled in the relevant art that various changes in form and details can be made therein without departing from the spirit and scope of the invention.

[0048] All references, issued patents and patent applications cited within the body of the instant specification are hereby incorporated by reference in their entirety, for all purposes.

ABSTRACT

Fluorescent nanoparticles, combustion-based methods for their synthesis, and methods for functionalizing nanoparticles are described. The methods provided by the invention are simplified, efficient and cost effective as compared to prior art methods. The resulting fluorescent nanoparticles have reduced tendancy toward aggregation, and diminished need for post-manufacturing processing steps.

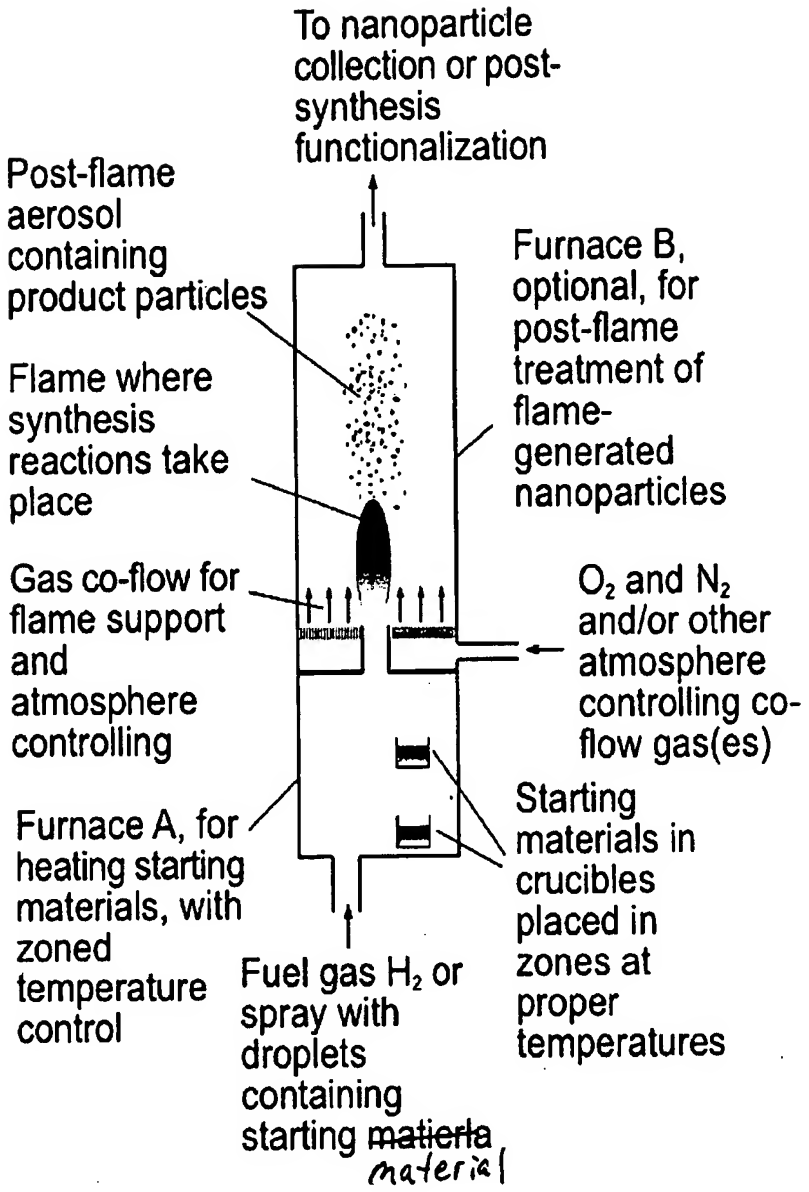

To nanoparticle collection or post-synthesis functionalization
Post-flame aerosol containing product particles
Flame where synthesis reactions take place
Gas co-flow for flame support and atmosphere controlling
Furnace A, for heating starting materials, with zoned temperature control
Fuel gas H_2 or spray with droplets containing starting ~~matierla~~ material
Furnace B, optional, for post-flame treatment of flame-generated nanoparticles
O_2 and N_2 and/or other atmosphere controlling co-flow gas(es)
Starting materials in crucibles placed in zones at proper temperatures

Figure 1

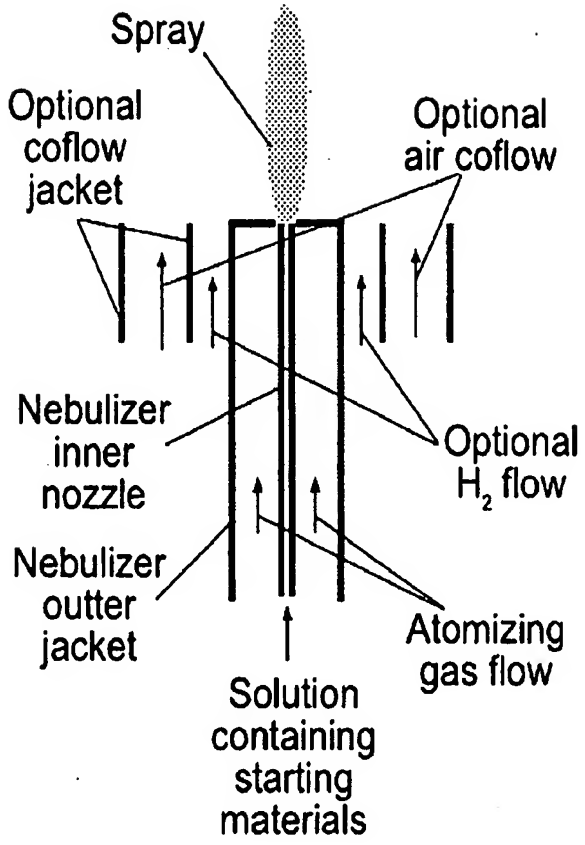

Spray
Optional coflow jacket
Optional air coflow
Nebulizer inner nozzle
Optional H_2 flow
Nebulizer outter jacket
Atomizing gas flow
Solution containing starting materials

Figure 2

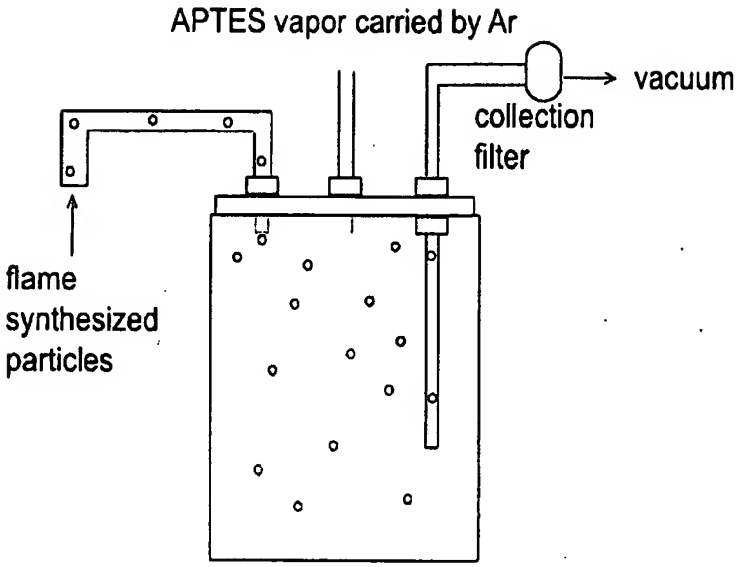
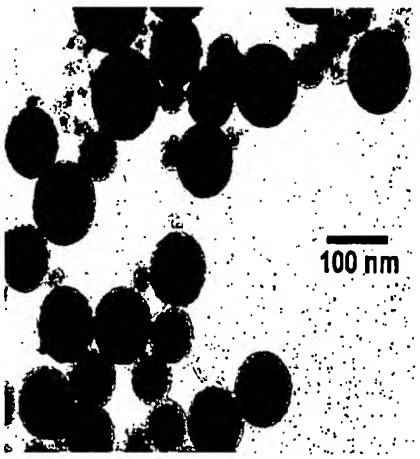
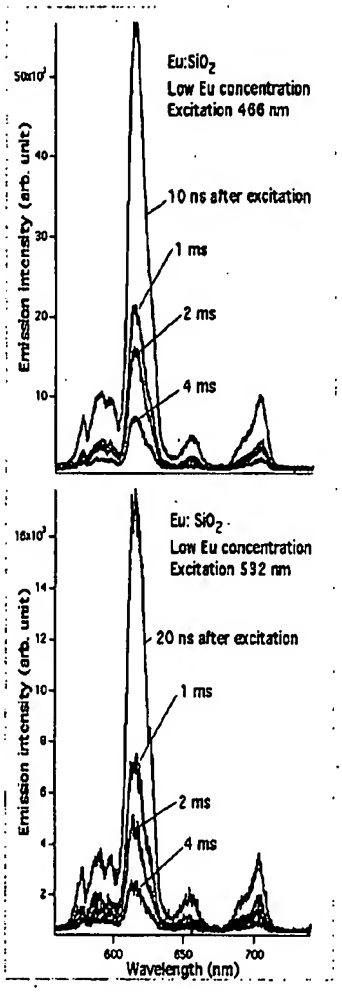

APTES vapor carried by Ar
vacuum
collection
filter
flame
synthesized
particles

Figure 3

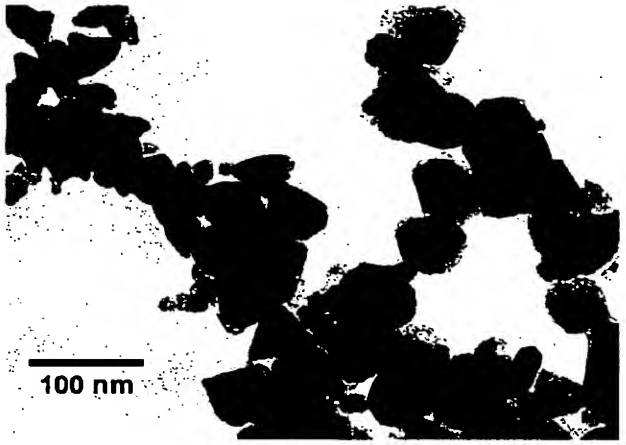


TEM Image, Eu-doped Silica Nanoparticles; Spherical, Non-aggregated

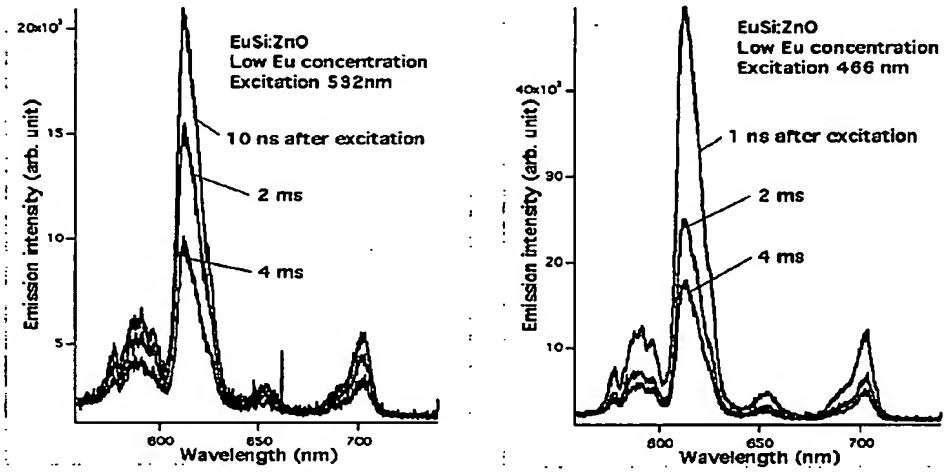


Fluorescence Spectra; Dominant Peak at ~ 615 nm; Lifetime ~ 2 ms; Convenient Excitation Wavelength at 532 nm

Figure 4

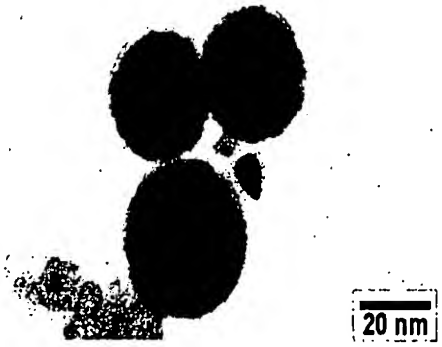


TEM Image; EuSi:ZnO Nanoparticles

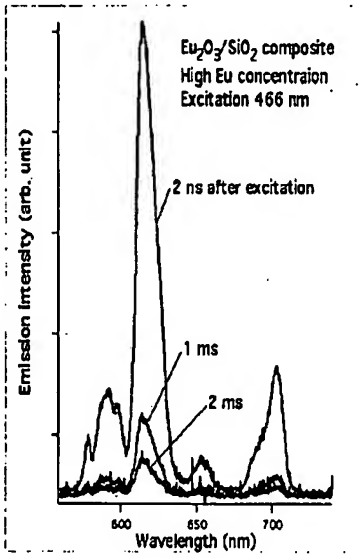


Fluorescence Spectra; Dominant Peak at ~ 615 nm; Lifetime ~ 4 ms; Convenient Excitation Wavelength at 532 nm

Fig. 5

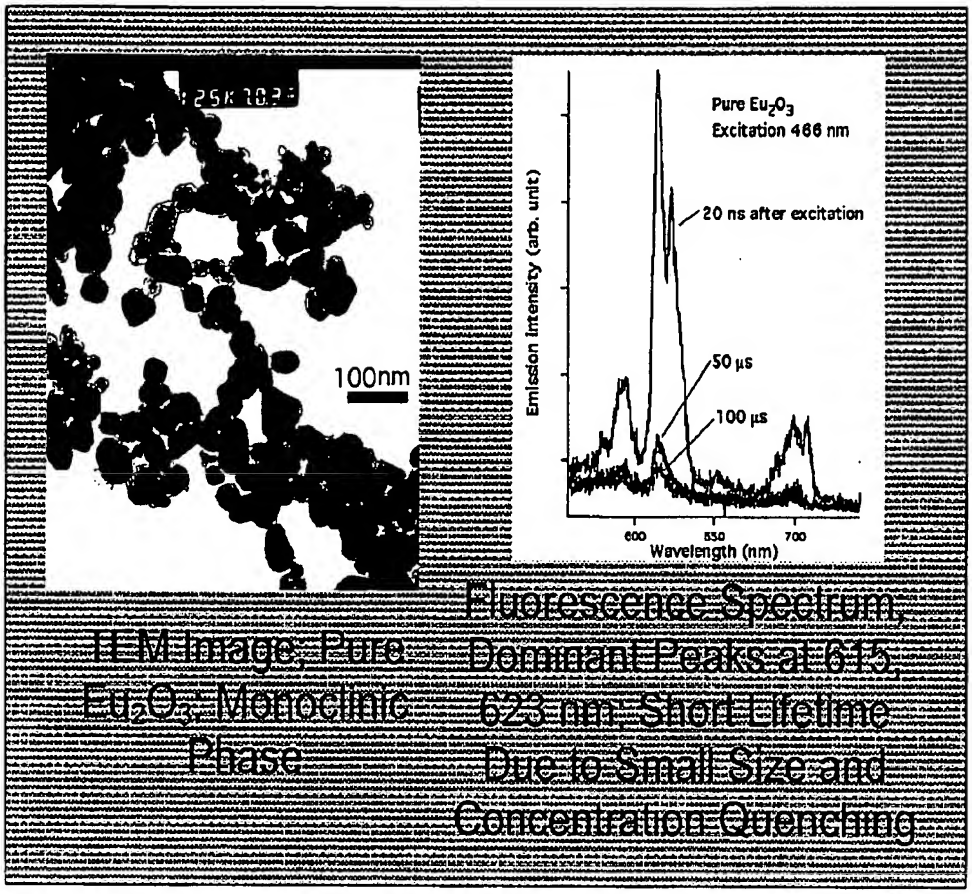


TEM Image; Eu_2O_3/SiO_2 Composite Nanoparticles; Higher-density Core with Lower-density Shell



Fluorescence Spectrum; Dominant Peak at ~ 615 nm; Lifetime ~ 1 ms

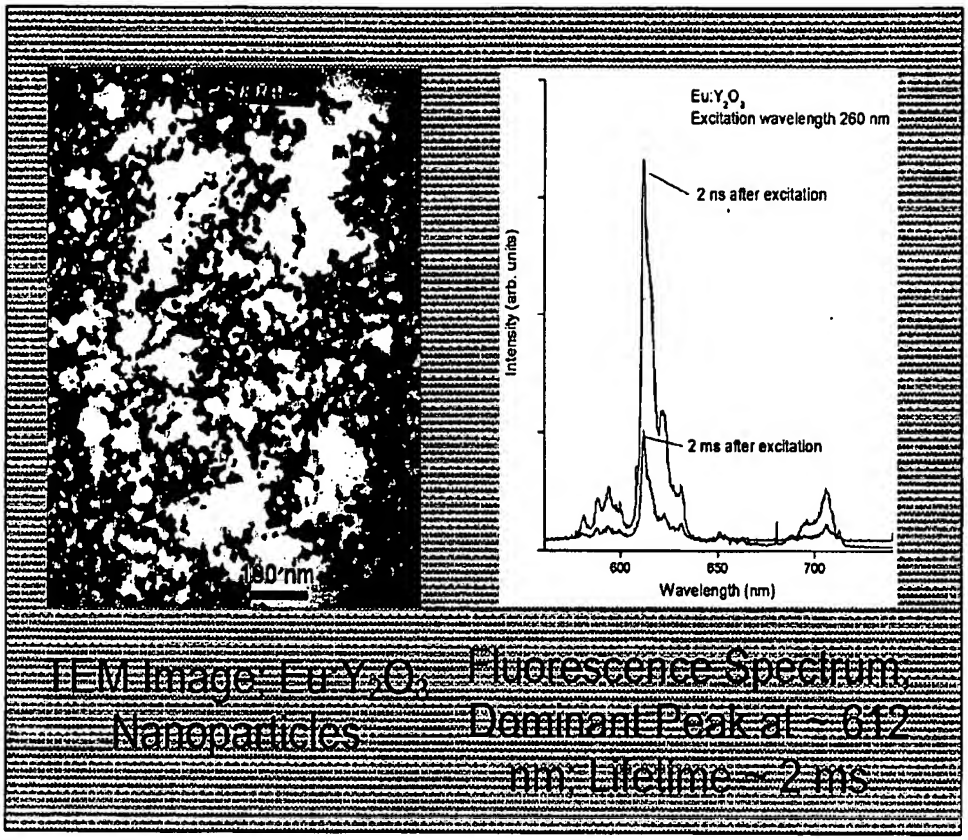
Figure 6



TEM Image: Pure Eu_2O_3: Monoclinic Phase

Fluorescence Spectrum, Dominant Peaks at 615, 623 nm; Short Lifetime Due to Small Size and Concentration Quenching

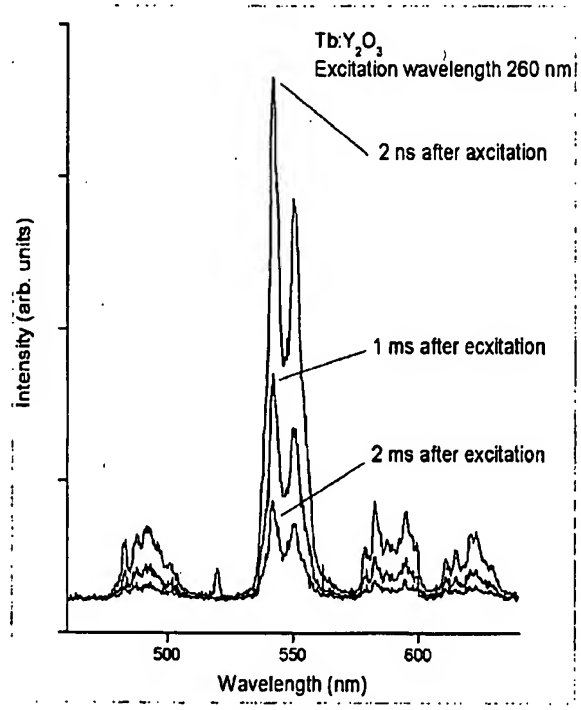
Figure 7



TEM Image: Eu:Y_2O_3 Nanoparticles

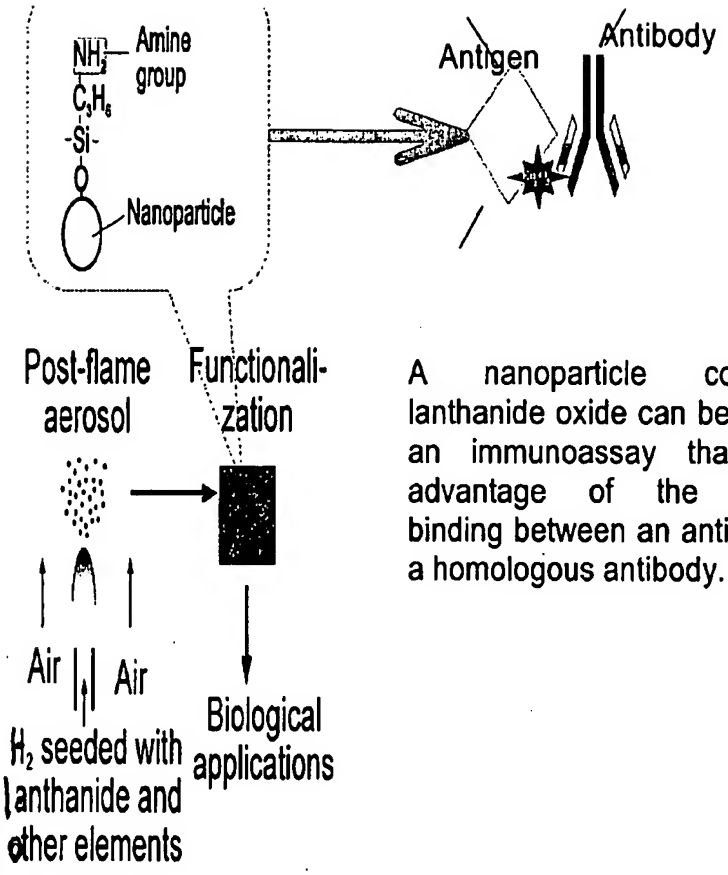
Fluorescence Spectrum, Dominant Peak at ~ 612 nm; Lifetime ~ 2 ms

Figure 8



Fluorescence Spectrum; Dominant Peak at 543 and 550 nm; Lifetime ~ 2 ms

Figure 9



A nanoparticle containing lanthanide oxide can be used in an immunoassay that takes advantage of the specific binding between an antigen and a homologous antibody.

Figure 10

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Title Line Two:: g Nanoparticles
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Formal Drawings?:: No
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Licensed US Govt. Agency:: NSF
Contract or Grant Number:: 0102662
Secrecy Order in Parent Appl.?:: No

REPRESENTATIVE INFORMATION

Representative Customer Number:: 758
Registration Number One:: 41310
Registration Number Two:: 47668

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